

# Selected Grassy Weeds as Alternate Hosts of Northern Corn Rootworm (Coleoptera: Chrysomelidae)

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**ABSTRACT** We evaluated the suitability of three grassy weed species, large crabgrass (*Digitaria sanguinalis* L. Scop.), giant foxtail (*Setaria faber* Herrm.), and barnyardgrass (*Echinochloa crus-galli* L. Beauv.), and a forage grass, tall fescue (*Festuca arundinacea* Schreb.), along with maize (*Zea mays* L.), as hosts of the northern corn rootworm, *Diabrotica barberi* Smith & Lawrence. Potted greenhouse plants were infested with northern corn rootworm eggs, and after hatch, larvae were recovered over time by using Tullgren funnels. The percentage of larvae recovered varied significantly between species at each sample date. The highest percentage of larvae were recovered from barnyardgrass, maize, and large crabgrass followed by giant foxtail and tall fescue. When sample dates were combined to test the main effect of species, there was no significant difference in the percentage of larvae recovered between maize and three other species (barnyardgrass, large crabgrass, and giant foxtail). The change in head capsule width (growth to new instars) also varied significantly between grass species on the second, third, and fourth sample date, but not the first. On the second sampling date, most of the larvae from maize were third instars, and their head capsule width was greater than the head capsule width of larvae recovered from any of the other species. Although the greatest percentage of larvae was recovered from barnyardgrass, larval development on this species was reduced, because average head capsule width of larvae recovered from all sample dates indicated they were mostly second instars. Average larval weights were not impacted by the grassy weed species evaluated. In a laboratory experiment, we studied the host-searching behavior of neonate larvae, in which the movement patterns were traced after contact with and removal from the roots of different species. In this study, we included the above-mentioned species, side-oats grama (*Bouteloua curtipendula* Michx. Torr), sorghum (*Sorghum bicolor* L.), and moistened filter paper, which served as a control. There were significant differences in the area searched, number of turns, path crossings, and the average velocity of the northern corn rootworm neonate larvae exposed to the different plant species and the control. The importance of the results in relation to resistance management is discussed.

**KEY WORDS** grassy weeds, alternate hosts, northern corn rootworm, *Diabrotica barberi*, host-searching behavior

THE NORTHERN CORN ROOTWORM, *Diabrotica barberi* Smith & Lawrence, is one of the major pests of maize, *Zea mays* L., in the midwestern and northern maize-growing regions of the United States, causing damage to maize by feeding directly on the roots (Chiang 1973). It is estimated that the loss to growers in terms of control costs and yield reduction caused by the corn rootworm complex (including the western corn rootworm, *Diabrotica virgifera virgifera* LeConte) is ≈\$1 billion annually (Metcalfe 1986). The primary means of protecting maize roots from corn rootworm species is

through the application of soil-applied insecticides or crop rotation (Krysan and Miller 1986). However, extensive use of these tactics has selected for resistance to several insecticides in the western corn rootworm (Ball and Weekman 1962, Meinke et al. 1998, Wright et al. 2000). Crop rotation also has selected for northern corn rootworm individuals that have an extended diapause and overwinter two or more years (Krysan et al. 1984, Krysan and Miller 1986), and western corn rootworm beetles that oviposit in crops adjacent to maize fields (Levine et al. 2002). The failure of these primary control strategies for both the northern and western corn rootworm has created a need that might be addressed by new technologies. Seed companies have developed maize hybrids containing genes that code for production of insecticidal proteins from the soil bacterium *Bacillus thuringiensis* Berliner (Bt) that have high levels of antibiosis to neonates of the rootworm complex. The U.S. Environmental Protection Agency has required a resis-

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tance management plan before registration of all Bt crops. One aspect that is important for the optimization of resistance management plans for rootworm-resistant transgenic maize is the role of alternate larval hosts in maize agroecosystems. Alternate larval hosts are important because larvae could potentially begin their development on a grassy weed and gain enough mass to overcome the dose of insecticidal protein expressed in the roots of transgenic maize (Hibbard et al. 2003, 2004; Clark and Hibbard 2004; Oyediran et al. 2004; Wilson and Hibbard 2004).

Northern corn rootworm larvae have been successfully reared on several grass species (Branson and Ortman 1967, 1971), but the majority of the species were evaluated only in petri dishes without soil. The northern corn rootworm is currently the dominant species in parts of Minnesota, South Dakota, and Iowa, and is common throughout much of the Corn Belt. The historical range of northern corn rootworm likely predates the introduction of maize into the region by native Americans and colonizing farmers of European descent (Galinat 1977, Branson and Krysan 1981) with northern corn rootworm larvae using unknown host plants (Webster 1986, Branson and Krysan 1981, Krysan and Smith 1987).

At least for high-dose products, movement of larvae from susceptible to transgenic plants, or vice versa, could adversely affect resistance management in several ways (Mallet and Porter 1992, Davis and Onstad 2000). Larger larvae are more tolerant to endotoxins and the initial development by these larvae on a susceptible plant (a grassy weed or maize) followed by subsequent migration to a nearby transgenic plant could accelerate the rate of adaptation if heterozygotes with the resistance gene survived exposure to the endotoxin at higher rates. Likewise, if larvae briefly fed on a transgenic root and then migrated to a nearby susceptible root, this too could accelerate the rate of resistance development if heterozygotes with the resistance gene were preferentially selected. Although there has been little published research on northern corn rootworm plant-to-plant larval movement, Hibbard et al. (2003, 2004) demonstrated that western corn rootworm larvae can move at least three plants down a row and across a 76-cm row. To develop a sound resistance management plan for the northern corn rootworm, additional information is required in a number of research areas, including the role of grassy weeds as alternate hosts and possible larval movement between alternate hosts and transgenic plants.

Apple and Patel (1963) examined the distribution of northern corn rootworm larvae within maize roots and determined that larvae were present primarily in centers of newer root tissues and the surrounding soil. As maize germinates, adventitious roots arise in whorls or nodes arising from the stem below ground (crown roots) and later above ground (brace roots). Survival of the larvae depends on their ability to locate and recognize these host roots. Search behavior in animals, in general, takes place in two phases: ranging and local search (Jander 1975). The former is characterized by relatively straight locomotion in which the animal has

limited resource information, and the latter is characterized by increased turning rate and decreased locomotor rate when animals perceive information of suitable resources (Bell 1985). According to Browne (1977), host selection by phytophagous insects can be considered as choice behavior with two extremes. The first extreme is that insects choose solely after contact with a potential host plant. In this case, the frequency with which different host and nonhost plants are visited depends on their relative abundance in the environment. The other extreme is that insects perceive plant characteristics of their host (e.g., volatile chemicals, color, and shape) at a distance and choose to visit the host because of these host-specific signals. These two extremes coincide with the distinction between what is called host plant recognition and host plant finding. Visser (1988) also pointed out that host plant finding is affected by both distribution of the host and the insect orientation. Many subterranean phytophagous insects locate hosts by host-oriented responses rather than by random movements (Calkins et al. 1967). In a larval host search behavior study with western corn rootworms, Strnad and Dunn (1990) demonstrated that search area and locomotor rate decreased and turning rate and path crossings increased after the larvae contacted host roots compared with contact with nonhost roots.

The goals of the current study were to evaluate selected grassy weeds for their ability to support northern corn rootworm development and to determine whether the host recognition bioassay developed by Strnad and Dunn (1990) for western corn rootworm larvae could be used for the northern corn rootworm.

## Materials and Methods

**Greenhouse Experiment.** We evaluated the suitability of three grassy weed species, large crabgrass (*Digitaria sanguinalis* L. Scop.), giant foxtail (*Setaria faber* Herrm.), barnyardgrass (*Echinochloa crus-galli* L. Beauv.), and a forage grass, tall fescue (*Festuca arundinacea* Schreb.), as larval hosts for the northern corn rootworm with maize serving as a control (Table 1). One maize kernel and 3 g of each weed species were planted in 3.8-liter clay pots containing 2:1 (vol: vol autoclaved) soil/peat-based growing medium mixture (Promix, Premier Horticulture LTEÉ, Québec, Canada). Drainage openings in the pots were fitted with a fine (114  $\mu$ m per opening) stainless steel mesh (TWP Inc., Berkley, CA) to prevent larval escape (Clark and Hibbard 2004). Plant species were in a split-plot arrangement of treatments in a randomized complete block design with four replications. For larval recovery, the main plot of the experiment was plant species and the subplots were sample dates. The experimental design for adult recovery was arranged as a randomized complete block with four replications (no split-plot). Five pots of each treatment were required for each replication (one for adult emergence and four for larval sampling times). Different sample times of the same treatment were randomized and

Table 1. List of plant species screened as hosts and used for northern corn rootworm larval search behavior study

Taxonomy of species evaluated <sup>a</sup>	Economic importance	Source
Family Poaceae		
Side-oats grama, <i>B. curtipendula</i>	Forage	Valley Seed Service, Fresno, CA
Subfamily Panicoideae		
Tribe Andropogoneae		
Maize, <i>Z. mays</i>	Crop	USDA-ARS, PGRU, Columbia, MO
Sorghum, <i>S. bicolor</i>	Crop/Weed	Pioneer Hi-Bred Inc., Johnston, IA
Tribe Paniceae		
Large crabgrass, <i>D. sanguinalis</i>	Weed	Valley Seed Service, Fresno, CA
Giant foxtail, <i>S. faberi</i>	Weed	Valley Seed Service, Fresno, CA
Barnyardgrass, <i>E. crus-galli</i>	Weed	Valley Seed Service, Fresno, CA
Subfamily Pooideae		
Tribe Poeae		
Tall fescue, <i>F. arundinacea</i>	Weed	Valley Seed Service, Fresno, CA

<sup>a</sup> According to the Germplasm Resources Information Network (GRIN), <http://www.ars-grin.gov/npgs/>.

placed adjacent to each other for a given replication. Each set of five pots for each treatment was randomly placed within each replication in the greenhouse. Five weeks after planting, each pot was infested with 40 northern corn rootworm eggs suspended in 1 ml of 0.15% agar solution by using a micropipette placed into a 2.5-cm-deep hole that was dug in each pot. After infestation, each hole was filled with soil. The source of the eggs was the USDA-ARS Northern Grains Insects Research Laboratory in Brookings, SD. Extra pots that did not form part of the experiment were infested and checked twice per week to determine timing of egg hatch. A photoperiod of 14:10 (L:D) h was maintained with 1000-W sodium bulbs (GE Lighting, Cleveland, OH). Temperature was maintained at  $27 \pm 2^\circ\text{C}$  as monitored with a temperature recorder (SL 4350C7C, Dickson, Addison, IL). At 5, 10, 15, and 20 d after egg hatch was thought to have peaked (as determined by sampling extra pots), the contents (soil mixture, roots, and larvae) of each pot randomly assigned to be sampled that date were placed in a Tullgren funnel equipped with a 60-W incandescent light bulb (Great Value, Wal-Mart, Bentonville, AR) for the extraction of larvae. Collection jars containing water were placed under the funnels and checked daily for larvae for 4 d. All northern corn rootworm larvae recovered from the collecting jars were transferred to labeled scintillation vials containing 95% ethanol. The number of larvae recovered from each pot was recorded and converted to a percentage of the 40 eggs infested (no adjustment was made for an unknown of actual viable eggs). The head capsule width of each larva was measured using an ocular micrometer (10 $\times$ /21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (M3Z, Wild Co. Heerbrugg, Switzerland), and the dry weights were determined after placing the larvae in a desiccating oven (Thelco model 16, GCA/Precision Scientific Co., Chicago, IL) at  $90^\circ\text{C}$  for 48 h. The change in head capsule width reported in Table 3 is the head capsule width of larvae recovered with the Tullgren funnel minus the head capsule width of neonate larvae (0.21 mm). On the 28th day after infestation, vegetation from the remaining pots (except maize) were trimmed to within 3 cm of the soil surface to allow the proper covering of insect mesh (0.06-mm

opening, ECONET L, LS Americas Co., Charlotte, NC) over each pot. Trimming did not kill any of the species evaluated, which continued to grow beneath the mesh. A hole in the mesh through which the maize plant grew was sealed with a cable tie. Each pot was checked a minimum of twice weekly for adult emergence. All individuals collected were immediately placed in 95% ethanol and stored until they were identified to sex, measured for head capsule width, and weighed as was described for the larvae.

**Laboratory Host Recognition Assay.** The effect of root contact on host-searching behavior of neonate larvae that had been starved for 12 h was evaluated after removal from the root using the methods of Strnad and Dunn (1990). Individual neonate larvae were treated by placing them onto roots of germinating (<5-d-old) seeds with a moistened camel's-hair brush. The species evaluated included maize, large crabgrass, giant foxtail, barnyardgrass, tall fescue, and side-oats grama (*Bouteloua curtipendula* Michx. Torr.). Sorghum (*Sorghum bicolor* L.) was used as a nonhost control (Branson and Ortmann 1967, 1971). Moist filter paper also was included as a control. After the 5-min treatment, larvae were transferred with a moistened camel's-hair brush to the center of an 18-cm-diameter circle of filter paper (Whatman, Pittsburgh, PA) that had been moistened with distilled water and placed on a glass plate (30 by 30 cm). A second glass plate was placed onto four 4-mm supports surrounding the filter paper. A transparency film (28 by 21 cm) was placed on the second glass pane. New moistened filter paper was used for each larva. Immediately after removal from the treatment and placing the larva on filter paper, the path traveled by each larva was traced on the transparency sheet with felt pen for 5 min. Area searched was later determined by superimposing the traced paths over a grid and counting the number of different squares (10 squares per 25 mm) intersected by the path. These values were then converted to square millimeters. The number of turns (defined as any abrupt change at  $\approx 90^\circ$  in the axis of travel) and the number of times that a larva crossed its own path also were determined. The distance (millimeters) traveled by neonates was determined using a curvimeter (RUN-MATE Instruments, Woubrugge, The Nether-

Table 2. Percentage of northern corn rootworm larval recovery (mean  $\pm$  SEM) from Tullgren funnels

Host species	Approximate days after median egg hatch				Species main effect
	5 d	10 d	15 d	20 d	
Maize	9.7 $\pm$ 5.6bA	9.7 $\pm$ 2.6bB	12.0 $\pm$ 2.9aB	12.6 $\pm$ 4.8aC	11.0 $\pm$ 2.4AB
Large crabgrass	6.8 $\pm$ 2.4bB	8.6 $\pm$ 2.3bB	15.4 $\pm$ 4.1aAB	13.7 $\pm$ 3.9aB	11.0 $\pm$ 2.3AB
Giant foxtail	6.3 $\pm$ 3.6aB	2.9 $\pm$ 2.9bC	2.3 $\pm$ 1.1bC	4.5 $\pm$ 3.2aD	4.0 $\pm$ 2.1BC
Barnyardgrass	6.9 $\pm$ 4.1cB	17.6 $\pm$ 6.1aA	16.6 $\pm$ 4.2bA	17.7 $\pm$ 8.4aA	14.5 $\pm$ 2.5A
Bronson tall fescue	0.0 $\pm$ 0.0bC	0.6 $\pm$ 0.5abD	0.6 $\pm$ 0.5abD	1.1 $\pm$ 0.6aE	0.6 $\pm$ 0.5C
Sampling date main effect	6.1 $\pm$ 0.0a	7.5 $\pm$ 0.0a	9.3 $\pm$ 0.0a	9.8 $\pm$ 0.0a	

Although untransformed data are shown, statistics were performed using arcsine  $\sqrt{x}$  data. Significant differences ( $P \leq 0.05$ ) among species within a column are indicated by uppercase letters. Significant differences ( $P \leq 0.05$ ) among sampling dates within a row are indicated by lowercase letters.  $N = 5$ .

lands). The distance traveled by each larva was divided by time of experiment (minutes) to calculate average velocity. The experimental design was a randomized complete block with eight treatments and replicated 30 times (six people with each person performing five replications). Individuals doing the tracing served as the blocks.

**Statistical Analysis.** The larval recovery data were analyzed as a randomized complete block split-plot design by using PROC GLM of the statistical package SAS (SAS Institute 1990). The model contained the main plot of species, the subplot of sampling time, and their interaction. Least significant differences (LSDs) ( $LSD, P = 0.05$ ) were calculated for intraspecific growth and recovery over times and interspecific growth and recovery at a given sample time as described by Steel and Torrie (1980) by using mean squares and degrees of freedom parameters from the PROC GLM procedure. A separate analysis was done for percentage larval recovery, head capsule width, and average larval weight. Adult data were analyzed as a randomized complete block design. Although non-transformed data are shown in the tables, all data were transformed by square root of  $(x + 0.5)$  or arcsine of  $\sqrt{x}$  for percentage larval recovery to meet the assumptions of the analysis (Snedecor and Cochran 1989). The data were transformed because the normal probability plot of residuals of larval recovery, head capsule width, area searched, larval turnings, path crossings, and velocities indicated that data were not normally distributed. The square root  $(x + 0.5)$  transformation was used because it is the most effective if values are small and it stabilizes the variance (Snedecor and Cochran 1989). The host search data were analyzed as a randomized complete block design with the six individuals that did the larval tracings treated as the blocking factor in the analysis of variance (ANOVA).

Results

**Larval Recovery.** The percentage of northern corn rootworm larvae recovered from the Tullgren funnels was significantly different among the grass species evaluated ( $F = 14.85$ ;  $df = 4, 60$ ;  $P < 0.0001$ ). Sample dates ( $F = 1.30$ ;  $df = 4, 60$ ;  $P = 0.2834$ ) and the interaction between species evaluated and sample

dates ( $F = 0.55$ ;  $df = 4, 60$ ;  $P = 0.8744$ ) did not significantly affect percentage of larval recovery. Across sample dates, the highest percentage of recovered larvae was from barnyardgrass ( $14.5 \pm 2.5\%$ ) and the lowest was from tall fescue ( $0.6 \pm 0.5\%$ ). Larval recovery trends within sample dates were generally similar to those observed with the species main effect (Table 2).

**Head Capsule Width of Larvae.** The head capsule width of larvae recovered varied significantly among grass species ( $F = 3.55$ ;  $df = 4, 29$ ;  $P = 0.017$ ). However, sample dates ( $F = 0.69$ ;  $df = 4, 29$ ;  $P = 0.5683$ ) and the interaction between grass species and sample dates were not significant ( $F = 0.80$ ;  $df = 4, 29$ ;  $P = 0.6297$ ), perhaps indicating that sampling was initiated later than desired. Head capsule width of larvae recovered from maize was significantly larger than head capsule width of larvae recovered from all other species on the second sample date (Table 3). The head capsule width of field-collected neonate northern corn rootworms ranged from 0.20 to 0.26 mm, whereas the second and third instars ranged from 0.30 to 0.40 and 0.44–0.56 mm, respectively (Hammack et al. 2003). On the second sampling date, the larvae recovered from maize had reached the third instar as indicated by a head capsule width of 0.54 mm. On average, larvae recovered from barnyardgrass, large crabgrass, and giant foxtail did not reach the third instar, even on the last sample date. Larvae recovered from tall fescue had reached the third instar on the third sampling (Table 3), but very few of these larvae were recovered (Table 2).

**Average Dry Weights of Larvae.** There was no significant difference in the average dry weights of larvae recovered among species ( $F = 0.13$ ;  $df = 4, 29$ ;  $P = 0.6809$ ). Sample date and the interaction between grass species and sample dates did not vary significantly ( $F = 1.33$ ;  $df = 4, 29$ ;  $P = 0.2838$  and  $F = 0.74$ ;  $df = 4, 29$ ;  $P = 0.6805$ , respectively). The highest average dry weight was recorded for larvae collected from large crabgrass 20 d after infestation. Average dry weights ranged between 0.05 and 1.01 mg.

**Adult Recovery.** Adult recovery was very low with four adults ( $1.00 \pm 1.00$  per pot) being recovered from maize. No adults were recovered from the grassy weeds.



Table 3. Average change in head capsule width in mm (mean  $\pm$  SEM) of northern corn rootworm larvae from an initial head capsule width of 0.21 mm

Host species	Approximate days after median egg hatch				Species main effect
	5 d	10 d	15 d	20 d	
Maize	0.21 $\pm$ 0.04cA	0.33 $\pm$ 0.05aA	0.29 $\pm$ 0.06bA	0.36 $\pm$ 0.06aA	0.29 $\pm$ 0.05A
Large crabgrass	0.23 $\pm$ 0.14aA	0.24 $\pm$ 0.04aB	0.15 $\pm$ 0.02bC	0.23 $\pm$ 0.03aB	0.21 $\pm$ 0.05B
Giant foxtail	0.19 $\pm$ 0.05bA	0.15 $\pm$ n/abC*	0.21 $\pm$ 0.02abB	0.24 $\pm$ 0.00aB	0.19 $\pm$ 0.02B
Barnyardgrass	0.21 $\pm$ 0.06aA	0.16 $\pm$ 0.04bC	0.17 $\pm$ 0.04bC	0.13 $\pm$ 0.03cC	0.16 $\pm$ 0.04C
Tall fescue	n/a**	0.20 $\pm$ n/abC	0.30 $\pm$ n/abA	0.35 $\pm$ 0.05aA	0.28 $\pm$ 0.05A
Sampling dates main effects	0.21 $\pm$ 0.07a	0.21 $\pm$ 0.03a	0.22 $\pm$ 0.03a	0.26 $\pm$ 0.03a	

Although untransformed data are shown, statistics were performed using square root ( $x + 0.5$ ) data. Significant differences ( $P \leq 0.05$ ) among species within a column are indicated by uppercase letters. Significant differences ( $P \leq 0.05$ ) between sampling dates a row are indicated by lowercase letters.  $N = 5$ .

\* When n/a follows a  $\pm$ , larvae were recovered from only one replication.

\*\* Larvae were not recovered from any replication.

**Host Search Behavior Patterns.** There was a significant difference in the area traversed by the neonate larvae that were exposed to the different treatment ( $F = 11.8$ ;  $df = 7, 203$ ;  $P < 0.0001$ ) (Table 4). Neonates exposed to and removed from moistened filter paper (control) traversed the greatest area and neonates exposed to and removed from maize roots traversed the smallest area, and there was no significant difference in the area searched by neonates exposed to maize and barnyardgrass (Table 4). Area searched was not affected by the individuals collecting the data ( $F = 2.04$ ;  $df = 5, 168$ ;  $P = 0.0760$ ), and the interaction between area searched and data collectors was also not significant ( $F = 1.34$ ;  $df = 5, 168$ ;  $P = 0.1967$ ).

The number of turns made by neonate larvae varied significantly after contact with the roots of different species ( $F = 4.02$ ;  $df = 7, 203$ ;  $P = 0.0004$ ). The most number of turns was recorded for neonate larvae that were exposed to maize, and the least number of turns was for larvae exposed to large crabgrass (Table 4). There was no significant difference between the number of turns made by larvae exposed to maize, barnyardgrass, and side-oats grama (Table 4). The number of turns was not affected by persons collecting the data ( $F = 1.86$ ;  $df = 5, 168$ ;  $P = 0.1038$ ); however, the interaction between turns and persons was significant ( $F = 1.87$ ;  $df = 5, 168$ ;  $P = 0.040$ ).

There was a significant difference in the number of path crossings made by neonate larvae exposed to the different treatments ( $F = 4.33$ ;  $df = 7, 203$ ;  $P = 0.0002$ ) (Table 4). The neonate larvae exposed to maize roots

had significantly more crossings than those exposed to any other root sources or the control (Table 4). Path crossing data were significantly affected by persons collecting the data ( $F = 4.27$ ;  $df = 5, 168$ ;  $P = 0.0010$ ), but the interaction between path crossings and persons was not significant ( $F = 1.12$ ;  $df = 5, 168$ ;  $P = 0.3067$ ).

There was also a significant difference between plant species in the velocity of travel by larvae exposed to the different treatments ( $F = 8.66$   $df = 7, 203$ ;  $P < 0.0001$ ). The highest velocity was recorded from larvae that were exposed to the control and the least was in maize (Table 4). There was no significant difference between the velocities of neonate larvae exposed to maize and barnyardgrass. The velocities of the neonate larvae were not significantly affected by individuals collecting the data ( $F = 1.14$ ;  $df = 5, 168$ ;  $P = 0.3397$ ) nor was the interaction between plant species and data collectors ( $F = 2.04$ ;  $df = 5, 168$ ;  $P = 0.0600$ ).

Discussion

Several seed companies have developed transgenic maize hybrids with strong antibiosis for the control of neonate corn rootworm larvae. The first of these products was registered for commercial use by the Environmental Protection Agency in February 2003. This product, YieldGard Rootworm, uses the Cry3Bb1 protein for corn rootworm control and can be stacked with glyphosate tolerance to allow for postemergence treatment of a broad spectrum of weeds. Cry3Bb1 is

Table 4. Area searched, number of turns, number of crossings, and velocity (mean  $\pm$  SEM) of neonate rootworms during 5 min after removal from contact with selected grassy weeds roots and control

Host species	Area (mm <sup>2</sup> ) <sup>a</sup>	Turns <sup>a</sup>	Crossing <sup>a</sup>	Velocity (mm/min) <sup>a</sup>
Maize	25.50 $\pm$ 3.77c	10.07 $\pm$ 1.13a	7.10 $\pm$ 1.66a	13.25 $\pm$ 1.80c
Large crabgrass	48.20 $\pm$ 5.57b	5.13 $\pm$ 0.66c	1.87 $\pm$ 0.59b	21.52 $\pm$ 2.72ab
Giant foxtail	58.57 $\pm$ 5.06b	5.40 $\pm$ 0.76bc	2.70 $\pm$ 0.76b	26.70 $\pm$ 2.36ab
Barnyardgrass	30.57 $\pm$ 4.93c	8.40 $\pm$ 1.3a	3.56 $\pm$ 0.94b	14.83 $\pm$ 2.21c
Bronson tall fescue	58.80 $\pm$ 5.31b	5.87 $\pm$ 0.73bc	2.37 $\pm$ 0.81b	26.57 $\pm$ 2.61ab
Side-oats grama	55.93 $\pm$ 5.05b	7.63 $\pm$ 0.87ab	2.30 $\pm$ 0.79b	25.17 $\pm$ 2.29b
Sorghum	56.40 $\pm$ 4.50b	5.87 $\pm$ 0.87bc	2.67 $\pm$ 0.59b	25.20 $\pm$ 2.16b
Moistened filter paper (control)	77.03 $\pm$ 5.44a	5.87 $\pm$ 0.73bc	3.10 $\pm$ 0.85b	35.59 $\pm$ 1.94a

Means followed by the same letter are not significantly different using LSD ( $P = 0.05$ ).

<sup>a</sup> Data were transformed using square root ( $x + 0.5$ ). Nontransformed means are presented.  $N = 30$ .

effective only against the first instars of the corn rootworm (EPA Scientific Advisory Panel 2002). Therefore, larvae that initially develop on grassy weeds and then move to rootworm-resistant, transgenic maize after the weed is killed may have increased chance of survival over larvae that did not initially establish on a grassy weed. If adoption of transgenic maize with tolerance to broad spectrum nonresidual herbicides becomes prevalent when stacked with rootworm-resistant, transgenic maize, grassy maize field weeds could become more important in the northern corn rootworm life cycle.

We recovered larvae from all the grassy weed species evaluated in this study. The highest percentage of larval recovery was from barnyardgrass and the least was from tall fescue. Results from the current study corroborate prior studies by Branson and Ortman (1967) that many grassy species in addition to maize are able to support the growth of northern corn rootworms. However, we found that barnyardgrass, which was listed as a nonhost to the northern corn rootworm larvae by Branson and Ortman (1967), was actually a better northern corn rootworm larval host in terms of percentage of larval recovery than giant foxtail, which was listed as host.

The larval head capsule width varied among grass species (Table 3). Interestingly, even though the highest percentage of larval recovery was from barnyardgrass (Table 2), this did not translate into larger average head capsule widths. Most of the larvae recovered on barnyardgrass, giant foxtail, and large crabgrass were second instars on all sampling dates (Table 3). These grassy weeds could be an inferior source of nutrition needed for the growth of the larvae compared with maize. Larvae recovered from maize were third instars on all but the first sample date. On the fourth sampling date, all larvae recovered from tall fescue were third instars, but very few larvae were recovered from this species.

Although adult emergence was very low, because the percentage of viable eggs was not known and not accounted for, adult emergence is comparable to previous greenhouse studies. If we assume that the highest percentage of larvae recovered is somewhat representative of the number of viable eggs, then only  $\approx 8$  larvae were infested in each pot. Given this, recovering only one adult per pot was a 12.5% recovery. These numbers are lower than Oyediran et al. (2004) (30%) but actually greater than Clark and Hibbard (2004) (4.5%) and Wilson and Hibbard (2004) (5.5%), all of whom used similar materials and methods to our experiment, but infested with live western corn rootworm larvae rather than northern corn rootworm eggs. It is not clear why adult emergence was so low in all of these studies. Perhaps the pot size was too small for a greenhouse environment. Perhaps roots of these species do not have the proper nutrition for adult emergence. Even maize, when infested very late, will support larval development without adult emergence (B.E.H., unpublished data). Regardless, the primary point of the greenhouse portion of this study was that northern corn rootworm larvae could at least

partially develop on grassy weeds. This provides that possibility that larger larvae could be migrating back to transgenic, rootworm-resistant corn when the weeds are killed.

In terms of search pattern (area traversed, number of turns, and velocity traveled), there was no significant difference between maize and barnyardgrass. Even though larval tracings were done by six different individuals, most factors were not significantly affected by individuals collecting the data. However, observations of crossing paths were significantly affected by the data collector. This may be because this particular parameter was more subjective than the other parameters.

Neonate northern corn rootworm larvae that contacted maize and barnyardgrass roots switched from ranging to localized searching. Our findings were similar to the findings of Strnad and Dunn (1990) on host search behavior in nondiapausing western corn rootworm. According to Strnad and Dunn (1990), restricted, highly convoluted searching increases the probability of encountering food within a food patch, whereas the straighter ranging behavior increases search efficiency between patches (Visser 1988). Our bioassay data indicate the at northern corn rootworm perceive maize and barnyardgrass as hosts.

Kogan (1982) pointed out that feeding behavior of polyphagous insects was divided into host habitat, host location, host recognition, host acceptance, and host suitability. The presence of carbon dioxide acts as an attractant and corn rootworm larvae are able to detect it (Strnad et al. 1986, Hibbard and Bjostad 1988). In our study, the neonate larvae had a localized search behavior after exposure and removal from maize roots. Contact host recognition cues are apparently used in the same way by northern corn rootworm larvae as western corn rootworm larvae. Strnad and Dunn (1990) stated that the occurrence of host roots within the soil may be patchy during the period when larvae hatch. Individual roots (food patches) are located relatively large distances apart when compared with the body size of the neonate northern corn rootworm larvae. Movement through certain soils is difficult for these larva, and delaying host finding by 24 h can increase larval mortality to 50% (Strnad and Bergman 1987). To survive, the neonate northern corn rootworm larvae must locate and remain in a food patch large enough to feed it and other larvae that find the same patch. The shift from ranging behavior (used to locate food patches) to local searching behavior (used to stay within the patch) after contact is apparently the method by which this is accomplished. Transition from localized searching back to ranging searching removes insects from food patches where continued search may energetically be unprofitable (Bell 1985). According to Nakamuta (1985), the duration of localized searching depends partly on the intensity of the feeding stimulus. In the soil, maize root quality decreases with time (Kessebalch 1949). Thus, to survive, larvae must leave older roots and infest younger, higher quality roots arising from the newer root whorls (Apple and Patel 1963, Strnad and Bergman 1987).

Because new root growth constitutes the formation of a new food patch distinct from the patch being abandoned, the shift to ranging behavior and location of new roots or food patches is of high survival value.

In summary, we examined the effect of selected grassy weeds on the survivorship, growth, and development of the northern corn rootworm. The results suggest that grassy weeds can have a significant impact on northern corn rootworm biology and that barnyardgrass and large crabgrass are better alternate hosts of the northern corn rootworm than giant foxtail and tall fescue (although none produced adults). The data support the potential of alternate host use by northern corn rootworm larvae in maize fields. However, additional studies are necessary to document whether movement from alternate hosts to rootworm-resistant, transgenic maize affects the selection intensity and fitness of northern corn rootworm in comparison with populations without partial development on alternate hosts.

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